The comparison of long and short primers used for RAPD technique in grape

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Abstract
In this research RAPD method was used with the help of individual selective short primers(10 mer)and long primers(15-21 mer).the number of produced polymorphic items ranged in order from 122 and 136 bands for short and long primers. In multiplication reaction , Items in the size area of 1904 to 564 pair base Resulted for short primers and 3530 to 564 pair base for long primers. In this study , by comparing the results gained from technique long and short primers in RAPD , the potential value of long primers for the production of polymorphism in grapes was indentified.

Key word : random primer, long primer , RAPD , polymorphic

Introduction
Developments in reforming plants and molecular biology and along with the protection of vital varieties in and out of the fields . Responses of responsibilities for long life protection has increased the usage of plant genetic sources . Unfortunately the budget for this work is less and the collection with respect to efficiency , preservation , assessment and determination of the properties and then use had not shown much efficiency . One of the possible solution is to use the cheap and beneficial technique in order to get the information for better organization of beneficial genetic variations present in collections .

These molecular techniques have maximal consistency with classic methods and can be used for the following tasks :

1- Distinguishing or separating the spices 4 characters from each other .
2- To measure the genetic variability .
3- To determine the genetic relationship .

Relationship between the markers with desired properties with respect to inte The numerous recent studies indicate the existence of a vast range of knowledge and high-amount budgets for the use of RAPD markers and so prove the adequacy of this
These developments are also helpful for a description of the genetic variety of plant productions and the most appropriate way for their use and protection (16). The RAPD (random amplified polymorphic DNA) technique (13, 15) has been widely used in plants for the construction of genetic maps in species such as *Arabidopsis* (10), bananas (3) and slash pine (8), and for genotype identification and taxonomic studies (2, 5).

RAPD markers are detected by the use of short oligonucleotides of arbitrary sequence as primers for the amplification of segments of the target genome. Generally, 10-mer primers with 50-80% G+C content are preferred. However, complex banding patterns were also generated with primers as short as 5 bases (4, 12, 13, 14). There are few reports on the use of long primers (over 12 bases). The potential value of long primers (15-21 bases) for generating RAPD polymorphisms was investigated in this study.

In this research not only the application of long primers in RAPD techniques in Biotechnology laboratory of agriculture department of Zabol university was started but the major task was also followed which comprises the comparision of long and short primers in RAPD techniques in IRAN.

**DNA extraction**

Grind 0.5 g of leaves using mortar and pestle in the presence of liquid nitrogen and their genomic DNA was extracted by protocol Lodhi et al (6). In this method for taking the polyphonys, PVP and for elimination of the consumed polysaccharides, Nacl was used. After that the DNA plate formed got soluble in TE Buffar. Quantity and quality of genomic DNA was assessed by biophotometry. In this order the basic solutions in which this proportion was between 1.8-2 was selected. After that the working solution or DNA with concentration of 10 nanogram was formed and this concentration of DNA was used in the laboratory.

**Amplification**

Genomic DNA was amplified using 50 different RAPD primers. The reaction included 50 mM KCL, 10 mM tris HCL (PH=8/0), 0/1% triton x-100, 2mM mgcl2, 200 µm each dATP, dCTP, dTTP, dGTP, 2unit taq DNA polymerase, 0.4 µm
primer and 10 ng genomic DNA, in a final volume of 25 μl. Cycling parameters were 40 cycles of 94 °C, 30s; 36 °C, 1min; and 72 °C, 2 min. After the last cycle, the samples were kept at 72 °C for 8 min and then cooled to 4 °C. PCR product were separated by electrophoresis on a 1.4 % agarose gel and visualized by ethidium bromide staining.

The selection of primers

Short primers were chosen from the primers used in RAPD technique in grape by Lodhi et al (7). The long primers from the primers used in RAPD research were selected by Lodhi et al(7) and the primers used by Dr. Mahmoud solooki(11).

Results and Discussion

Generally, 10-mer primers with 50-80% G+C content are preferred (7) in RAPD analyses. As the length of a primer increases, the genomic target sites should decrease because there is less chance of finding perfect or near perfect homologies between the target sites and a longer primer(9). For the accomplishment of this study, 50 long and short primers were used from which 21 of the primers produced identified electrophasic bands.

Accidental primers with the lengths of 21, 20, 18, 17, 16, 15 and 10 nucleotids were applied by RAPD marker. Long primers have also been used in genomic fingerprinting. The primers with 16 nucleotids, produced 78 percent of polymorphic, the primers with 17-18 nucleotids produced 87 percent and the primers with 20 to 21 nucleotids produced 100 percent of these bands. While, the primers with 10 to 15 nucleotids produced in order 68 and 70 percent of polymorphic bands. We observed, however, that long primers yielded more polymorphic bands than the short 10-mer primers that this will be increase efficiency of RAPD.

Typical gels displaying the amplification products generated from grape DNA using long primers are shown in Fig. 1. the long primers generated more DNA fragments, a wider range of DNA fragment sizes (typically 564-3530 Pb vs. 564-1904 Pb for 10-mer primers) and a greater number of polymorphic fragments per primer (Table 1).

The results of this research are approved by the studies of Guang Ning and et al(4) in 1996 on grapes, Masumi et al., (12) in 2002 on Asiatic hybrid lily and Barysheva et al., (1) in 1995 on grape.
long primers used in this study have G+C content from 39% to 55% and short primers have G+C content more of 50%. It is not clear in our study whether the increased number of bands produced by long primers was due to lower G+C content, primer length, or a combination of both. Future experiments should be designed to determine the effects of primer length and G+C content upon RAPD polymorphisms in a variety of genera.

The results of this research are approved by the studies of Guang Ning and et al., in grapes, they stated that if the generation of more fragments is due to primer length, one possibility could be that the extra bases at the 5’ end anneal to the templates in a way that either helps the 3’ bases anneal to the template to start a new template-primer complex or stabilizes the unstable existing template-primer complex. Because most of the primers have less than 50% G+C content, intergenic or repetitive DNA regions may be preferentially targeted, which could be useful in mapping telomere and centromere regions that are otherwise not as easily accessible with 10-mer primers. Sequencing of the amplified fragments or probing of the genomic DNA with the amplified fragments would help to determine the nature of amplified products and the mechanism(s) underlying the amplification.

The higher cost for synthesizing long primers can be justified by the greater number of polymorphic bands obtained. Long primers might also be available gratis from colleagues in a large research facility. To generate the same number of polymorphic bands, reactions with 10-mer primers cost up to three times as much in materials and labor as reactions with long primers.

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References


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Table 1. The effect of primer length (10 to 21 bases) on number of RAPD fragments and number of polymorphic fragments per primer

<table>
<thead>
<tr>
<th>Primer Length (Bases)</th>
<th>No. of Primers Tested $a$</th>
<th>Average total no. of Fragments/Primer</th>
<th>Average no. of polymorphic Fragments/Primer</th>
</tr>
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<tbody>
<tr>
<td>10</td>
<td>9</td>
<td>122</td>
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Figure 1. RAPD profiles of grape DNA generated with the 20-mer CO4
TGCCCTCCATTCGTAGCCAA